Data Requirement: US EPA DP Barcode: 399085
398866
US EPA Guideline: OPPTS 850.6100

Test material:
Common name: Ethaboxam
chemical name:
IUPAC: N-(α-cyano-2-thenyl)-4-ethyl-2-(ethylamino)-5-thiazolecarboxamide
CAS name:
CAS No: 162650-77-3
synonyms**:**

Primary Reviewer (officer number): Hélène Arsenault (2077)
PMRA

Secondary Reviewer: **Date: 11/13/13**

Andrew Shelby
EPA

Signature:

Company Code:	VAJ
Active Code:	EBX
Use Site Category:	10, 11
EPA PC Code:	090205

Part 8.2.2 Analytical methodology (parent compound and transformation products)

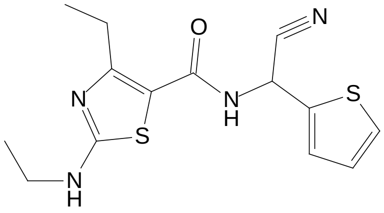
Common Name:	Ethaboxam
Product Name:	Ethaboxam Technical
Submission Number:	2011-4730
PCPA Reg. Number:	Not yet assigned
Source Code:	EBX-LGS-2

Chemical structures:

Table 1. Chemical name, code and chemical structure for active and all major transformation products / metabolites

Chemical name	Code	Chemical structure

Table 1. Chemical name, code and chemical structure for active and all major transformation products / metabolites

N-(cyano-2-thienylmethyl)-4-ethyl-2-(ethylamino)-5-thiazolecarboxamide	LGC-30473 (parent)	

No major transformation products are expected.

Data Submission and Review History:

Table 2. Correspondence Dates, Data # and Content for Ethaboxam Technical

Date Received	Data #	Content Summary	Reviewer Officer #
03 Nov 2011	1	Parts 8.2.2.1 and 8.2.2.2 study reports	1930

<u>Good Laboratory Practices Compliance Statement</u>:

The studies contained within this report were conducted in accordance with the Good Laboratory Practice Standards as specified in 40 CFR 160.

yes [x]   no [ ]   not stated / applicable [ ]

Note: The original method validations do not specify GLP compliance, although the test facility is accredited by the UK monitoring authority and a study director was named and study protocol written. The ILV study was EPA GLP compliant.

8.2.2 Analytical Methodology (parent compound and transformation products)

8.2.2.3 Water

Reference:

1) PMRA # 2111121. EPA MRID 48535671. 2003, VALIDATION OF METHODOLOGY FOR TNE POST REGISTRATION MONITORING OF RESIDUES OF LGC-30473 IN DRINKING, GROUND AND SURFACE WATER, DACO: 8.2.2.3 (Data # 1)
2) PMRA # 2111120. EPA MRID 48535625. 2011, Independent Laboratory Validation for the Determination of V-10208 Residues in Soil and Water, DACO: 8.2.2.1,8.2.2.3 (Data # 1)

Note: A second analytical method (with validation data) for fresh and salt water was provided in PMRA # 2138203, EPA MRID 48695901. This HPLC-UV method was less sensitive (LOQ 40 µg /L) than the method detailed below, and was considered a secondary method to the one reviewed below.

Table 5. Principle of the method (LKF 115)

Items	**Details**
Details of sample used	Original validation: drinking water, surface water and ground water ILV: Untreated tap water
Extraction method used	A sample of water (100 mL) is transferred into a 250 mL separatory funnel (sample is fortified at this point if required). Dichloromethane 25 mL (or 50 mL for surface water) is added and the sample shaken for ~ 1 minute. The dichloromethane layer is transferred to a 100 mL round bottom flask, and the aqueous layer is re-extracted with an additional 25 mL (or 50 mL for surface water) dichloromethane as before. The combined extracts are evaporated to dryness using rotary evaporation at 40 °C, and the residues are reconstituted in 20 mL methanol : water (50:50). Samples may be further diluted as required to be in calibration range.

Table 5. Principle of the method (LKF 115)

Items	**Details**
Method for identification and quantitative analysis of parent compound and transformation products	HPLC-MS (Electrospray positive ion mode) Instrument: HPLC with binary pump Detector: MS/MS Column: Phenomenex Luna C-8 15 cm x 2.0 mm i.d., 5µm particle size Mobile Phase: A) 80:20 (v/v) water : acetonitrile B) 20:80 (v/v) water : acetonitrile (both containing 0.01 M ammonium acetate / 0.1 % acetic acid) Gradient: Time (min) % A %B Flow 0.00 60 40 0.2 mL/min 6.00 0 100 0.2 mL/min 12.00 0 100 0.2 mL/min 13.00 60 40 0.2 mL/min 25.00 60 40 0.2 mL/min Retention time: 7.2 min. (LGC-30473) Cycle time: 25 min. MS conditions: Ionization mode: ESI (positive ion mode) Acquisition type: MRM MRM (Q1) 321.00 → (Q3) 200.00 m/z $M^+ \rightarrow [M - 121]^+$ (probable loss of C_6H_3NS (α-cyano-2-thienyl), as the main peak in the MS (EI+) spectrum is 183 m/z
Chromatograms of spiked sample, control sample, blank and standard solution	Chromatograms of standard solutions, spiked (fortified at 1.0 and 10.0 µg/L) samples and control samples (ground, surface and drinking water) were provided in the original study. Chromatograms of standard solutions, spiked (fortified) water samples and a control water sample were provided in the ILV study.
Quantitation	By method of external standards using linear regression on a 10 level (9 level for ILV) standard curve.
Criteria for setting LOD and LOQ	The LOQ was defined as the lowest fortification level at which acceptable recovery data were obtained. The LOD was defined as the equivalent sample concentration of the lowest calibration standard chromatographed.
Stability of parent and transformation products at various stages of analysis	The stability of the parent at various stages of the analysis was not discussed. Recoveries of the active indicate that there were no stability issues in the timeframes used.
Special problems encountered and/or precautions to be taken during analysis/handling/storage of samples	No specific problems or matrix effects were noted.
Total time for completion	6 hours to complete an extraction set of 12 water samples

The method validation data for the parent compound are summarized in Table 4.

Table 6. Method validation: Parent compound

Parameter	**Parent compound**			
	Validation study surface water [1]	**Validation study ground water** [1]	**Validation study drinking water** [1]	**ILV study untreated tap water** [2]
% Recovery at spiking level-1 (n = 5 at 0.1 µg/L)	____	88 (6.6 %RSD)	82 (11.7 % RSD)	____
% Recovery at spiking level-2 (n = 5 at 1.0 µg/L)	91 (3.0 % RSD)	95 (9.7% RSD)	87 (11.7 % RSD)	109 (2.95 %RSD)
% Recovery at spiking level-3 (n = 5 at 10.0 µg/L)	94 (5.0 % RSD)	____	____	109 (2.79 % RSD)
Mean % recovery	93 (n = 10)	91 (n = 10)	84 (n = 10)	109 (n = 10)
RSD %	4.3	8.8	11.5	2.6 [4]
Method linearity	0, 1 – 50 ng/mL (9 levels and a blank)	0, 1 – 50 ng/mL (9 levels and a blank)	0, 1 – 50 ng/mL (9 levels and a blank)	1 – 50 ng/mL (9 levels, forced through zero)
Correlation coefficient	0.9993	0.9993	0.9993	≥ 0.9988
LOD	0.2 µg/L	0.02 µg/L	0.02 µg/L	0.2 µg/L [3]
LOQ	1.0 µg/L	0.1 µg/L	0.1 µg/L	1.0 µg/L

[1] data from Ref. 1 (pg 15- 17, 26-28)
[2] data from Ref. 2 (pg 20, 25)
[3] based on the sample concentration equivalent to the lowest standard prepared – judging by the LOQ spike and unspiked control chromatograms, the actual LOD was estimated to be 30 – 50 times lower than this value (pg 28,29)
[4] calculated by reviewer

Conclusions/Other Comments: An HPLC-MS/MS method was developed for the determination of ethaboxam in water and was validated in four water types. The recovery data were acceptable (between 70-120%), and the LOQ was determined to be either 0.1 µg/L or 1.0 µg/L. This method is acceptable for use as a post-registration monitoring method.

Overall Summary of Data

Part 8 Environmental Chemistry and Fate

Matrix	Method	Fortification level (n)	Parent compound		LOQ	Method accept-ability
			Mean recovery (%)	RSD (%)		
Sandy Loam soil	LFK114	0.05 mg/kg	91	6.3	0.05 mg/kg	A
		0.5 mg/kg	100	6.3		
Clay Loam soil		0.05 mg/kg	100	4.8		
		0.5 mg/kg	92	5.3		
Loamy Sand soil		0.05 mg/kg	101	3.7		
		0.5 mg/kg	101	2.4		
Sediment	The method for soil can be extended to sediment – no additional data were provided					A
Surface water	LFK115	1.0 µg/L (5)	91	3.0	1 µg/L	A
		10 µg/L (5)	94	5.0		
Ground water		0.1 µg /L (5)	88	6.6	0.1 ug/L	
		1.0 µg /L (5)	95	9.7		
Drinking water		0.1 µg /L (5)	82	11.7	0.1 ug/L	
		1.0 µg /L (5)	87	11.7		
Tap water		1.0 µg/L (5)	109	2.9	1 µg/L	
		10 µg/L (5)	109	2.8		
Plant	reviewed by HED.					
Animal matrix						N

Conclusion: The analytical methods developed for determination of ethaboxam in soil, sediment, and water have been validated and determined to be acceptable as post-registration monitoring methods. Methodology for the active ingredient in animal biota (preferably birds) is required by EAD and is still outstanding. This deficiency is included in the EAD level C deficiency memo, PMRA # 2144906.